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Delayed Administration of Sodium Thiosulfate in Animal Models Reduces Platinum Ototoxicity without Reduction of Antitumor Activity[1]

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ABSTRACT

Platinum-based chemotherapeutic agents, such as carboplatin and cisplatin, are effective against many human tumors, but their use may be limited by a high incidence of ototoxicity. Delayed administration of the chemoprotective agent sodium thiosulfate (STS) reduces the ototoxicity of carboplatin in a guinea-pig model, when given up to 8 h after the chemotherapy, and also reduces hearing loss in patients given carboplatin with osmotic blood-brain barrier opening for treatment of brain tumors. We tested whether STS, given at times that achieved otoprotection, could impact the chemotherapeutic efficacy of carboplatin. The impact of STS was evaluated by measuring the onset of growth of LX-1 human small cell lung carcinoma s.c. xenografts in the nude rat. When STS was administered as two boluses, 2 and 6 h after treatment with carboplatin and etoposide, there was a decrease in the time to tumor progression. In contrast, when STS administration was delayed until 8 h after carboplatin/etoposide, there was no reduction in the antitumor cytotoxicity of the chemotherapy. STS infusion did not significantly affect ultrafilterable platinum pharmacokinetics in the guinea pig. To explore the potential wider applicability of STS, in a pilot study we tested its efficacy against cisplatin ototoxicity. Delayed administration of STS, 2 h after cisplatin, was protective against cisplatin-induced ototoxicity in the guinea pig model, as determined by electrophysiological measures. On the basis of these data, we suggest that delayed administration of STS may provide a mechanism to reduce the ototoxicity caused by administration of carboplatin or cisplatin for both central nervous system and systemic cancer chemotherapy.

INTRODUCTION

The platinum-based chemotherapeutic agents carboplatin and cisplatin have a broad spectrum of activity against systemic malignancies (1, 2), and carboplatin is also effective against brain tumors, if delivery to the tumor is maximized with osmotic BBB[3] opening (1). Cisplatin and carboplatin have largely equivalent therapeutic efficacies against many solid tumors, but they differ widely in their toxicity profiles (3, 4). A major toxicity of cisplatin therapy, nephrotoxicity, may be largely avoided with adequate hydration and diuresis, whereas the major hematological toxicities of carboplatin may be reduced with bone marrow growth factors. With the reduction in traditional toxicities, peripheral neurotoxicity and cochlear toxicity are dose limiting for cisplatin (3, 4). Hearing loss has not been considered previously as a major toxicity of carboplatin, but it is clear that with increased delivery, dose escalations, or with standard doses in sensitive populations, carboplatin is ototoxic (1, 5, 6, 7). Platinum-induced hearing loss is progressive and largely irreversible in patients, and animals models have demonstrated that the ototoxicity involves loss of the inner and outer hair cells of the inner ear (8).

Ototoxicity has a significant negative impact on patient quality of life. Mechanisms to decrease this undesirable effect would increase the use of the effective platinum-based chemotherapeutic agents. A possible solution to the problem of platinum ototoxicity may be the use of a chemoprotective agent (9). At high molar excess, STS binds to, and inactivates, cisplatin and carboplatin *in vitro* (10, 11), and STS reduces cisplatin nephrotoxicity in animal models (12) and in patients (13, 14). We have explored STS as an otoprotective agent. In a guinea pig model, we previously showed that high doses of STS reduced carboplatin-mediated cochlear damage, as confirmed by electrophysiological measurements and histology (8). In patient studies, blood concentrations of STS equivalent to those in the guinea pig model could not be achieved due to transient hypernatremia and hypertension (15). Nevertheless, significant otoprotection was demonstrated when STS was given 2–8 h after

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[3] The abbreviations used are: BBB, blood-brain barrier; ABR, auditory brainstem response; AUC, area under the curve; CSF, cerebrospinal fluid; STS, sodium thiosulfate.

administration of carboplatin with osmotic BBB disruption in brain tumor patients (15).

A possible drawback to the use of a chemoprotectant is its potential to interact with and reduce the desired antitumor effects of carboplatin or cisplatin. Two-route therapy has been used in studies of cisplatin and STS to provide high local antitumor activity with systemic chemoprotection (12, 13, 14). For example, the combination of i.p. administration of high-dose cisplatin with i.v. administration of STS has shown favorable results against ovarian cancer, allowing dose escalation while decreasing the nephrotoxicity of the drug (13, 14). Studies of STS in brain tumor patients made use of the two compartments generated by the presence of the BBB. Carboplatin was delivered to intracerebral tumors with transient osmotic BBB disruption, whereas STS, administered after the BBB permeability returned to normal, was used to reduce the systemic toxicity and ototoxicity of the drug remaining in the peripheral circulation (15).

Another alternative to avoid interactions of the platinum chemotherapeutic and the chemoprotective agent is to separate their administration in time. Previous studies of STS with cisplatin showed that the STS had to be administered within 5 min of the cisplatin to be effective at reducing nephrotoxicity (9, 13, 14). In contrast, STS was effective at reducing carboplatin ototoxicity when administered up to 8 h after carboplatin in the guinea pig (8). In the clinical brain tumor trial described above (15), STS was separated from carboplatin both by two compartments, and by time, in that STS was administered after the BBB closed, at 2 h after carboplatin.

The current studies were undertaken to determine whether the delayed administration of STS impacts carboplatin antitumor efficacy. Additionally, we evaluated the potential for delayed STS rescue of cisplatin ototoxicity. Our goal is to reduce the ototoxicity of platinum chemotherapeutics without a decrease in tumor cytotoxicity.

MATERIALS AND METHODS

Reagents. Carboplatin (Paraplatin), cisplatin (Platinol), and etoposide phosphate (Etopophos) were obtained from Bristol Meyers-Squibb (New York, NY). STS and D-methionine were obtained from Sigma Chemical Co. (St. Louis, MO).

s.c. Tumor Efficacy Studies. Animal studies were performed in accordance with guidelines established by the Oregon Health Sciences University Committee on Animal Care and Use. The LX-1 human small cell lung carcinoma cell line (originally obtained from Mason Research Institute, Worcester, MA) was maintained as a free-floating cell suspension in spinner flasks, in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Irvine Scientific, Santa Ana, CA) plus gentamicin, penicillin, and streptomycin. Cell suspensions with viability >90% by trypan blue exclusion and packed cell volumes of 20% ± 1% (~1×10^5 cells/μl) were used for tumor implantation. Adult, female nude rats from a colony maintained at Oregon Health Sciences University and weighing 220–240 g were briefly anesthetized with nitrous oxide and isoflurane inhalant (Isothesia; Abbott Laboratories, North Chicago, IL), and 600 μl of LX-1 cell suspension were inoculated into the s.c. tissue of the right flank.

Therapy studies were initiated 24 h after tumor cell inoculation. The rats were anesthetized with 2% isoflurane for placement of an i.v. catheter in the femoral vein, then anesthetized with propofol (650 μg/kg/min; Zeneca Pharmaceuticals, Wilmington, DE) as a constant infusion during the remainder of the treatment, as described previously (16). The right external carotid artery was surgically exposed and catheterized for infusion of carboplatin (200 mg/m^2) and etoposide phosphate (150 mg/m^2). This drug administration protocol mimics the regimen routinely used in brain tumor patients undergoing BBB disruption chemotherapy (1, 15). STS was given i.v. at a dose of 8 g/m^2. The experimental conditions were: (1) no treatment (n = 20); (2) carboplatin + etoposide phosphate (n = 20); (3) carboplatin + etoposide, followed at 2 h and at 6 h with STS (n = 8); and (4) carboplatin + etoposide, followed in 8 h with STS (n = 8).

STS Pharmacology Studies. Five male American shorthair outbred guinea pigs were anesthetized with sodium pentobarbital (30 mg/kg) and given STS at a dose of 11.6 g/m^2. STS was given as an i.p. bolus (n = 2) or as a 15-min i.v. infusion in the femoral vein (n = 3). Sixteen female Long Evans rats weighing 220–260 g were anesthetized with sodium pentobarbital (50 mg/kg) and given STS at doses ranging from 6–11.6 g/m^2. STS was administered either i.p. (n = 6) or as a 15-min i.v. infusion in the femoral vein (n = 10). Blood and urine samples were collected immediately after the i.v. infusion, or at 15 min (guinea pigs and rats) or 30 min (rats) after the i.p. bolus dose of STS.

Four dogs were given a 15-min infusion of 10% STS i.v. at rates that provided doses of 20 g/m^2 (n = 2), 30 g/m^2 (n = 1), or 40 g/m^2 (n = 1). In the dogs, serum was collected for determination of STS concentrations, acid-base status, and sodium and potassium concentrations during the infusion, immediately after, and 30 min after infusion. Urine was collected between 5 and 20 min after STS infusion and assayed for STS. In one dog, CSF was collected 4 h after STS infusion and assayed for STS concentration. Additionally, continuous electrocardiograms (n = 4) and noninvasive blood pressure monitoring (n = 2) were performed in the dogs during and after the STS infusion. All blood, urine, and CSF samples were evaluated for STS concentration using the methylene blue method, as described by Ivankovich *et al.* (17).

Carboplatin Pharmacokinetic Study. Guinea pigs were given carboplatin (24 mg/kg), followed at 1 h by furosemide (100 mg/kg), and followed at 2 h by either STS (11.6 g/m^2, n = 3) or saline (n = 3). Blood samples (0.5 ml each) were collected 5 min after each drug administration, as well as at 30 min, 1 h, 2 h, 3 h, 4 h, and 6 h after STS, with fluid replacement at each withdrawal time point. Plasma was prepared by centrifugation for 10 min at 3000 × g and 4°C and was stored at −70°C until analyzed for both total and ultrafilterable platinum. Ultrafiltrates were prepared using Amicon Centrifree micropartition devices (Amicon Division, WR Grace, Beverly, MA) with centrifugation at 2000 × g for 20 min at 4°C. Platinum concentration in plasma and ultrafiltrate was assessed with a Perkin-Elmer model 1100 flameless atomic absorption spectrometer (Perkin-Elmer Corp., Norwalk, CT) following a method validated in our laboratory and described previously (18). This method is similar to the platinum measurements described by Saito *et al.* (19).

Guinea Pig Ototoxicity Study. American shorthair outbred guinea pigs, weighing ~400 g and having an active Preyer pinna reflex, were used for the ototoxicity study (n = 4). To allow i.v. infusion of experimental agents, an indwelling polyethylene catheter (PE 50) was inserted into the left external jugular vein of each animal. The animals were anesthetized with pentobarbital (32 mg/kg i.p.), the surgical site was clipped of hair and scrubbed with Betadine, and the surgery was performed using sterile technique. After catheter placement, the surgical site was closed with Michelle wound clips. All guinea pigs were given cisplatin (6 mg/kg), followed after 1 h by furosemide (100 mg/kg). At 2 h after cisplatin, two animals were given STS (11.6 g/m^2) and two were given saline. The i.v. catheter was then removed, and the animals were returned to the animal care quarters where they were maintained for 8 weeks to allow the drug effects to stabilize.

Both ears of each animal were tested electrophysiologically, but only one ear from each saline animal was evaluated. Each guinea pig was anesthetized using i.p. allobarbital (60 mg/kg) and urethane (240 mg/kg). An endotracheal tube was inserted, and the animal was mechanically ventilated. Body temperature was maintained at 38.5°C, as monitored with a rectal thermistor probe. Scalp electrodes were placed to record ABR thresholds. For measurements of the compound action potential (N_1) threshold, the middle ear was exposed and a small silver ball electrode was placed on the round window membrane, as previously reported (8). ABR and N_1 thresholds were determined at six different frequencies from 2 KHz through 32 KHz.

Data Analysis. For the s.c. tumor growth study, tumor volume was determined daily by caliper measurements, using the formula: volume = width^2 × length/2. The delay between tumor implantation and the appearance of a measurable tumor, recorded as tumor day after implantation, was determined for each treatment group. This time to tumor progression was compared by one-way ANOVA using the JMP statistical program (SAS Institute Inc, Cary, NC). Comparisons for all pairs were made with Student's t test using the Tukey-Kramer procedure. The probability of appearance of measurable s.c. tumors was also assessed nonparametrically by the product limit (Kaplan-Meier) method, using the JMP statistical software. For STS pharmacokinetic studies, blood and urine STS concentrations were measured as described previously (8, 17), and means and SEs were determined for each species. In the carboplatin pharmacokinetic study, serum total platinum was comparable with ultrafilterable platinum concentrations for all animals and time points, therefore, only ultrafilterable platinum values are presented. Platinum concentration × time data for individual guinea pigs were analyzed using noncompartmental methods. Area under the concentration × time curve (AUC) was calculated with the log trapezoidal method. Clearance of ultrafilterable platinum was calculated from the equation: Clearance = dose/AUC. For the guinea pig hearing studies, mean N_1 thresholds and ABR measurements were recorded and means and SDs were compared by Student's t test at each frequency, and with each ear treated as an independent measurement.

RESULTS

Effect of STS on Carboplatin Cytotoxicity against LX-1 Tumors. Nude rat s.c. tumors grown from the LX-1 human small cell lung carcinoma cell line were used to investigate the effect of STS on antitumor efficacy. As a sensitive assay of chemotherapeutic efficacy, we assessed impairment of s.c. tumor growth (*i.e.*, time to progression) rather than decrease in size of an established tumor or animal survival. This assay maximized the effect of the carboplatin chemotherapy, and thus increased the possibility of observing a negative impact of STS on efficacy. A preliminary test showed that the LX-1 tumor model required the addition of etoposide phosphate with the carboplatin for significant antitumor activity. Etoposide phosphate alone had no impact on tumor growth (data not shown).

When animals were treated with carboplatin and etoposide phosphate 24 h after implantation of the tumor cells, a 60% increase was observed in the time to tumor progression. The delay between tumor cell inoculation and detection of a measurable tumor increased from 5.5 ± 0.4 days in the untreated control animals (n = 20), to 8.9 ± 0.6 days in the chemotherapy animals (n = 18, P = < 0.001; Fig. 1*A*). A product-limit (Kaplan-Meier) plot of the results for s.c. tumor growth in the rat model demonstrated that the carboplatin/etoposide treatment significantly decreased the probability of detecting tumor growth at early days after implantation (Fig. 1*B*). Treatment with STS at 2 h plus 6 h after carboplatin decreased the antitumor effect of the chemotherapy (Fig. 1*B*, *Line 3*). The time delay to a measurable tumor was 6.4 ± 0.8 days (n = 8) if animals received the two early boluses of STS, which is significantly below the treatment with chemotherapy alone (P = 0.012) and not significantly different from the untreated tumor growth (P = 0.164). Delay of the STS administration to 8 h after carboplatin/etoposide resolved much of its negative interaction with the chemotherapy (Fig. 1*B*, *Line 4*). Administration of STS 8 h after the combined carboplatin and etoposide phosphate treatment allowed significant prolongation of time to tumor detection (8.1 ± 0.7 days, n = 8, *versus* no treatment 5.5 ± 0.4 days, n = 20, P = 0.0023) and did not significantly reduce the efficacy of the drug treatment compared with chemotherapy alone (8.1 ± 0.7 d, n = 8, compared with 8.9 ± 0.6 days, n = 18, P = 0.188; Fig. 1*A*).

STS Comparative Pharmacokinetics across Species. The STS comparative pharmacokinetic data across species is shown in Table 1. Serum STS concentrations in guinea pigs and rats were assayed before and 15 min after an i.p. bolus or after a 15-min infusion of STS. When assessing all animals, regardless of route of administration, rats attained serum STS concentrations of 504 ± 106 mg/dl (n = 5), whereas guinea pig STS concentrations were 564 ± 70 mg/dl (n = 5) after receiving 11.6 g/m^2 of STS, as reported previously (8). STS was very rapidly excreted by the kidneys, and high STS concentrations were documented in the urine of all rats and guinea pigs, as reported previously (8). No STS was detectable in the serum of these animals before STS administration (n = 11). In both guinea pigs and rats, serum STS values were more variable and generally lower if measured 15 min after the i.p. bolus, as compared with immediately after the 15-min i.v. infusion (Table 1). When rats given 6 g/m^2 of STS were evaluated at 30 min after the i.p.

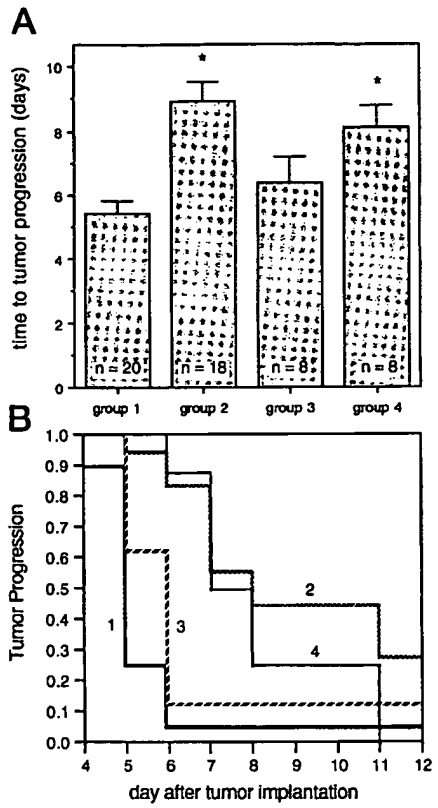

Fig. 1 Effect of STS and carboplatin on s.c. tumor growth. Nude rats were inoculated s.c. with 6×10^7 LX-1 tumor cells and were treated as: (1) no treatment (n = 20); (2) carboplatin (200 mg/m^2) + etoposide phosphate (150 mg/m^2; n = 18); (3) carboplatin + etoposide phosphate + STS (8 g/m^2) given 2 h and 6 h after carboplatin (n = 8); and (4) carboplatin + etoposide phosphate + STS (8 g/m^2) at 8 h after carboplatin (n = 8). s.c. tumor volume was measured daily, and the time to tumor progression was determined. *A*, days before detection of a measurable (~1 cm^3) s.c. tumor. Each *bar* indicates the mean ± SE for each experimental condition. *B*, product-limit analysis of the time delay before s.c. tumor progression for each experimental condition. For example, in group 1, 90% of animals had no detectable tumor on day 4, whereas on day 6, only 5% had no detectable tumor.

bolus, serum STS was increased to 144 ± 147 mg/dl (n = 4), demonstrating slower uptake of STS via the i.p. route. Doses of STS ranging from 6–8 g/m^2 were used in the rat to achieve serum STS levels similar to those attained in humans (330 mg/dl; Ref. 15).

STS was administered i.v. to four dogs at either 20 g/m^2 (n = 2), 30 g/m^2 (n = 1), or 40 g/m^2 (n = 1), resulting in peak plasma STS concentrations of 258, 404, and 564 mg/dl, respectively (Table 1). Mild to moderate hypernatremia (154–170 mEq/L) and mild hypokalemia (2.26–3.46 mEq/L) occurred in all dogs and were more pronounced with increasing STS dose. No STS was detected in the CSF of the one dog tested at 4 h after STS infusion.

Effect of STS on Carboplatin Pharmacokinetics in the Guinea Pig. The effect of STS on serum concentrations of platinum in the guinea pig was analyzed to determine whether the chemoprotectant might bind to and eliminate circulating carboplatin. Guinea pigs were given carboplatin (24 mg/kg), followed 1 h later by furosemide (100 mg/kg), and followed at 2 h by either STS (11.6 g/m^2, n = 3) or saline (n = 3), to mimic the ototoxicity induction/protection regimen used in previous experiments. Blood samples collected after each drug administration and throughout the clearance period were evaluated for ultrafilterable and total platinum concentration. The plasma concentration of ultrafilterable platinum in guinea pigs with and without STS administration are shown in Fig. 2. No significant effect of STS on ultrafilterable platinum concentration was found in these animals. One animal showed a rapid drop in serum ultrafilterable platinum at the point taken 5 min after the addition of STS, but serum platinum returned to near pre-STS infusion levels by 30 min after infusion of STS. Serum platinum concentrations were near zero by 8 h after carboplatin administration, with or without STS addition (Fig. 2).

To evaluate carboplatin clearance, the area under the platinum concentration × time curve (AUC) was determined for each animal, using the log trapezoidal method. The AUC was variable between guinea pigs, as shown in Table 2. No difference in clearance was found between animals given saline (184 ± 44 ml/hr/kg) compared with animals given STS (208 ± 51 ml/h/kg; P = 0.33).

Effect of STS on Cisplatin Ototoxicity. Cisplatin was ototoxic in the guinea pig when administered with furosemide, in the same paradigm described in previous studies for carboplatin ototoxicity (8). We performed a pilot test of otoprotection with STS in this model. One h after cisplatin, furosemide was administered, and 2 h after cisplatin, two animals received STS (11.6 g/m^2) and two animals received saline. Both saline-treated animals demonstrated marked auditory damage at each frequency tested between 4 KHz and 32 KHz (Fig. 3). Animals treated with delayed administration of STS maintained N_1 thresholds and auditory brainstem responses within the normal range throughout this frequency range (Fig. 3). Both electrophysiological measures of guinea pig hearing had equivalent results. Thus, STS prevented the 40–50 decibel increase in hearing threshold caused by cisplatin, even when administered 2 h subsequent to the chemotherapeutic agent.

DISCUSSION

STS and Carboplatin Cytotoxicity. Carboplatin, in conjunction with etoposide phosphate, maintained antitumor activity against LX-1 human small cell lung carcinoma s.c. tumors in the presence of delayed administration of STS. STS can have an antagonistic effect on the chemotherapeutic activity of carboplatin/etoposide phosphate against s.c. tumors in the rat, if the STS is given too soon after the platinum agent. The reduction of antitumor activity was avoided by delaying administration of the chemoprotectant until 8 h after chemotherapy. Although some interactions between the chemoprotectant and the platinum chemotherapy agent may not be detected with the s.c. tumor assay, these results are promising for the future use of STS in conjunction with therapy of systemic cancers.

In patients, the maximum tolerated dose of STS (20 g/m^2) yields a serum STS concentration approximately equal to that achieved in the rats with 8 g/m^2 (15). A 4-h separation for bolus deliveries is tolerable in patients. Given these dosing and timing

Table 1 STS comparative pharmacokinetics across species
Blood and urine samples were taken immediately after the 15-min i.v. infusion or at 15 min after the i.p. bolus.

Species	STS dose (g/m²)	Plasma STS (mg/dl)		Urine STS (mg/dl)
		i.v. infusion	i.p. bolus	
Guinea pig	11.6	666 ± 28, $n = 3$	411 ± 103, $n = 2$	1658 ± 660, $n = 5$[a]
Rat	6	281 ± 16, $n = 2$	33 ± 36, $n = 4$	
	8	343 ± 25, $n = 3$		
	11.6	581 ± 163, $n = 4$	196 ($n = 1$)	3861 ± 209, $n = 6$[a]
Dog	20	258 ± 37, $n = 2$		3383 ± 793, $n = 2$
	30	404 ($n = 1$)		2015 ($n = 1$)
	40	564 ($n = 1$)		3105 ($n = 1$)

[a] Values previously reported (8) are shown for comparison. Values indicate single measurements (for $n = 1$), means ± difference (for $n = 2$), or mean ± SD for the indicated number of animals.

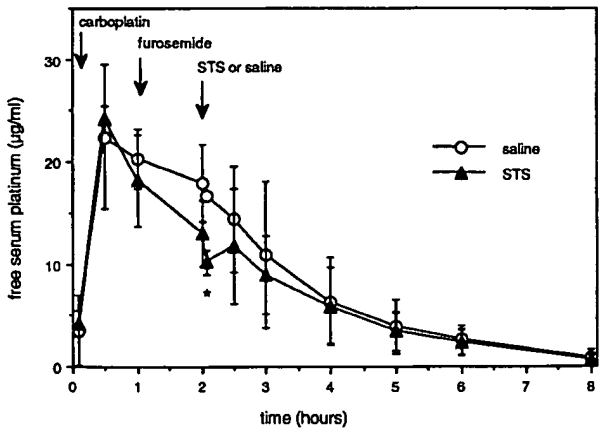

Fig. 2 Effect of STS on platinum pharmacokinetics in the guinea pig. Guinea pigs were treated with carboplatin [24 mg/kg (~184 mg/m²)], furosemide (100 mg/kg i.v.), and either STS (11.6 g/m²; ▲) or saline (○) at the time indicated by the *arrows*. Blood samples were obtained at the indicated times and analyzed for ultrafilterable platinum concentration. Note that the 2-h sample was collected before STS and the 2-h, 5-min sample was collected immediately after STS administration. Each point indicates the mean ± SD for $n = 3$ animals, except the sample obtained 5 min after STS (*), which represents two STS samples and one saline control.

constraints, several possible timing regimen approaches are possible to delay the STS after the chemotherapy, including the two investigated in this study (a relatively short delay plus a long delay *versus* a very long delay). The more aggressive regimen (2+6 h STS) markedly reduced the efficacy of chemotherapy, making it inappropriate for otoprotection after platinum chemotherapy treatment. A single dose of STS administered with an 8-h delay did not impact tumor treatment, making it an exciting approach for patient treatment.

The possibility of reduced anticancer effect in the presence of chemoprotective agents is a major concern limiting their applicability (9). Any reduction in chemotherapy concentration or activity may result in a decrease in the efficacy of the carboplatin or cisplatin. Results from other researchers are conflicting on this subject. Jones and Basinger (20) found no loss of cisplatin activity in rats treated with glutathione, used as a thioester in the same manner as STS. Iwamoto *et al.* (12) found that the addition of STS in two-route chemotherapy actually gave superior antitumor effects against mouse tumors. In contrast, Inoue *et al.* (21) found a decrease in the antitumor efficacy of cisplatin with the addition of STS. They found that administration of STS even 72 h after cisplatin significantly depressed cisplatin activity (21). This contrasts with our study, which showed no significant loss of carboplatin activity when STS was administered 8 h after chemotherapy. The reasons for these differences are not known.

Potential for Chemoprotection against Ototoxicity. Hearing loss is a serious side effect of chemotherapy with the platinum drugs and can have a negative impact on the quality of life that patients face after therapy. Ototoxicity is a major toxicity of cisplatin and is also recognized as an increasing problem with caboplatin therapy. Treatment protocols that increase the delivery of carboplatin to the cochlea may be associated with increased incidence of ototoxicity. For example, when carboplatin was delivered across the BBB with osmotic BBB disruption for treatment of brain tumors, high-frequency hearing loss was detected in 79% of patients (1, 15). Additionally, sensitive populations, such as children, may be more likely to suffer carboplatin ototoxicity. A recent study assessed ototoxicity in children treated with standard-dose carboplatin for neuroblastoma (7). Significant high-frequency hearing loss was found, and only 2 of 11 patients had no ototoxicity (7). In studies in which ototoxicity is not rigorously screened with serial audiometric testing, self-reported or anecdotal assessment of hearing loss may underestimate the problem of high-frequency hearing loss.

The results described herein confirm and expand our results, indicating that STS dramatically reduced the level of auditory damage due to administration of platinum. Our previous work in the guinea pig model demonstrated that carboplatin-induced ototoxicity could be completely prevented by treatment with STS as late as 8 h after carboplatin (8). In the current study, the protective effect of STS against platinum-induced hearing loss was documented in guinea pigs treated with cisplatin and furosemide. The previous results with carboplatin in the guinea pig model led us to test the potential for STS otoprotection in patients. We demonstrated a significant reduction in the magnitude of hearing loss after one treatment with carboplatin in patients receiving STS (3.7 ± 2 decibel, $n = 15$) in comparison with the historical comparison group (20.8 ± 5.9 decibel, $n =$

Table 2 Effect of STS on ultrafilterable platinum pharmacokinetics in guinea pigs given carboplatin

The AUC for each guinea pig was estimated from the concentration x time data with the log trapezoid method, and clearance was calculated as the platinum dose (12614 μg Pt/kg)/AUC. STS-treated animals were not significantly different than saline controls (P = 0.33).

	Saline			+STS	
	AUC (μg/ml) × h	Clearance ml/h/kg		AUC (μg/ml) × h	Clearance ml/h/kg
Guinea pig A	93.2	135	Guinea pig B	83.2	152
Guinea pig C	54.2	233	Guinea pig E	47.4	266
Guinea pig I	58.1	217	Guinea pig G	51.5	245
Mean ± SD	68.5 ± 21.5	195 ± 52	Mean ± S.D	60.7 ± 19.6	221 ± 61

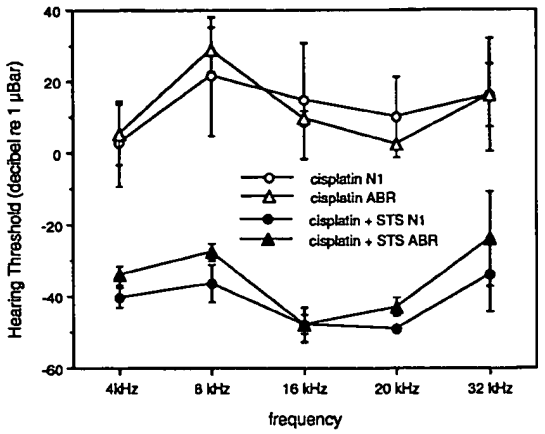

Fig. 3 Effect of STS on cisplatin ototoxicity. Guinea pigs were treated with cisplatin [6 mg/kg (~48 mg/m^2)], followed 1 h later by furosemide (100 mg/kg). Two h after cisplatin, the animals received either STS (11.6 g/m^2, ▲ and ●; n = 4 ears/2 animals) or saline (△ and ○; n = 2 ears/2 animals). After 8 weeks, ABR (▲ and △), followed by N_1 thresholds (● and ○) were obtained at the indicated frequencies.

19; Ref. 15). The reduction of cisplatin ototoxicity by STS in the guinea pig model supports the possibility that STS may be effective against cisplatin in patients as well.

Several attempts have been made to reduce the toxicities of cisplatin by using chemoprotective agents, although few of these studies targeted ototoxicity. Amifostine (Ethyol, WR2721) has been evaluated clinically as a chemoprotective agent with potential otoprotective activity (22). However, whereas a decrease in peripheral neurotoxicity was demonstrated, it is unclear whether otoprotection was found. In one clinical study, grade 3 ototoxicity occurred in 3 of 25 patients given amifostine in conjunction with cisplatin therapy (23), whereas in another study 3 of 13 patients with metastatic breast carcinoma displayed ototoxicity with a similar regimen (24). In the presence of another chemoprotective agent, diethyldithiocarbamate, ototoxicity incidence remained at ~50% and became the dose-limiting toxicity (25). The amino acid D-methionine is a thioether that has been proposed for otoprotection against the platinum chemotherapeutics (26, 27). In animal models, D-methionine effectively reduced the hearing loss caused by high-dose cisplatin (26), but this agent has not yet been tested for otoprotection in patients. In a preliminary study in the rodent LX-1 s.c. tumor model, we found that pretreatment with D-methionine did not significantly reduce time to tumor progression compared with chemotherapy with carboplatin plus etoposide phosphate (7.8 ± 0.5 days, n = 8 compared with 8.9 ± 0.6 days, n = 18, P = 0.070).

Many of the previous studies using two-route chemotherapy with the combination of high-dose i.p. cisplatin and i.v. STS demonstrated that chemoprotection for nephrotoxicity necessitated the addition of STS either concurrently with cisplatin or within 5 min of the chemotherapy (12, 13, 14). The 2- to 8-h timing for STS administration in the current study represents a significant delay. This timing difference (*i.e.*, 8 h) may be key to reducing the undesirable ototoxic effects of carboplatin while maintaining a high therapeutic effect.

Mechanism of STS Rescue of Carboplatin Toxicity. *In vitro*, STS binds directly to the electrophilic platinum, producing an inactive complex (10, 11). The molar ratio of STS to platinum agent is a primary determinant of the extent and rate of neutralization of platinum, and optimal ratios of STS have been shown to be 400:1 for cisplatin and 40:1 for carboplatin (10, 11). It is not clear whether this chemical neutralization is the mechanism of STS chemoprotection *in vivo*.

In vivo, the deactivation of cisplatin or carboplatin will depend on both the concentration of STS achieved in the serum and its timing related to cisplatin or carboplatin. In our animal studies, i.v. administration of STS was superior, in confirmation of results by Iwamoto *et al.* (12). In patients, serum STS levels could not be elevated as high as in the animals because of hypernatremia and hypertension, but the levels achieved were effective for otoprotection (15). The high serum STS concentrations achieved in our animal studies did not seem to alter plasma ultrafilterable platinum pharmacokinetics. One caveat is that the ultrafilterable platinum likely includes platinum bound to STS, which could not be distinguished from ultrafilterable platinum that was not bound to STS by the atomic absorption assay used. The presence of STS-platinum conjugates could have reduced the amount of active drug available, although an alteration in platinum pharmacokinetics was not detected. This lack of effect of STS on serum platinum concentrations confirms findings by Saito *et al.* (19) using a similar platinum assay.

A delay between administration of platinum drug and the subsequent administration of STS makes use of the clearance of these drugs to reduce the concentration of free drug available to interact with STS. It would then be easier to attain a high molar ratio of STS to platinum for deactivation of both remaining free drug as well as drug bound to cellular targets. This would be especially beneficial in patients because lower maximum serum STS concentrations can be achieved (15). When STS is administered at 8 h after carboplatin, a time point at which it remains

otoprotective, we found that serum platinum concentrations were near zero. It, thus, seems unlikely that reducing the remaining free carboplatin concentrations can account for the chemoprotective effect of STS. Although the mechanism of STS otoprotection at the molecular level is unknown, we hypothesize that there is direct interaction with the hair cells of the cochlea, to rescue them from carboplatin that has already bound to cellular targets. For example, STS may reduce platinum-DNA adducts, or restore the activity of DNA repair enzymes (28).

Potential Use of STS in the Clinical Setting. Given the incidence of high-frequency hearing loss associated with chemotherapy with the platinum agents, particularly cisplatin, a mechanism to decrease ototoxicity could be important for maximizing dosing and compliance and, therefore, chemotherapy efficacy. Delayed administration of STS is currently under assessment in brain tumor patients given enhanced delivery of carboplatin for treatment of central nervous system tumors (15). We suggest that a clinical trial to evaluate delayed administration of STS may be warranted to extend the positive results achieved in brain tumor patients, both children and adults, to systemic tumors being treated with platinum chemotherapy.

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